a novel inhibitor of matriptase shows good selectivity for matriptase compared to other trypsin-like serine proteases.
Conclusions: Building on evidence that matriptase acts as a novel initiator of cartilage collagen destruction, these new data show that it can also potently degrade cartilage proteoglycan by induction of MMPs and ADAMTS4. We have demonstrated that this breakdown is largely due to the activity of MMPs. The matriptase inhibitor appears to be potent and selective towards matriptase and represents a valid start point for drug development in OA.

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GLYCAN MAPPING AND GALECTIN-1 EXPRESSION IN OSTEOARTHRITIC CARTILAGE

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Purpose: The glycosylation of cells and tissues is a complex process that contributes to the adhesion capacity of cells and potentially affects cellular signal transduction or the onset of apoptosis. In this context, the glycan-encoded information may be translated into a biological effect by endogenous lectins such as galectins.
Despite the significance of glycoproteins for the maintainance of cartilage functionality, the potential implications of the chondrocyte glycophenotype in the pathophysiology of osteoarthritis (OA) remain largely unknown.
The present study aimed to assess the glycan profile and the expression of Galectin-1 in human OA cartilage and to determine the effect of Galectin-1 on primary chondrocytes in vitro.
Methods: Cartilage specimens were collected from OA patients undergoing knee replacement surgery (n=10) prior to processing for immunohistochemistry. Following staining with safranin-O, the grade of cartilage degeneration was defined in 90 histological sections using the Mankin degeneration score. Using a panel of 11 plant lectins, common constituents of natural glycans, serving as ligands for endogenous lectins (galectins), were attributed to the tissue sections. Histochemical analysis of Galectin-1 binding sites was accomplished using biotinylated human Galectin-1. The number of Galectin-1 positive chondrocytes was evaluated in cartilage specimens as a function of the degeneration score. The biological effect of Galectin-1 on isolated primary human chondrocytes was determined using RT-qPCR and ELISA assays.
Results: The intensity of plant lectin staining was subject to the grade of cartilage degeneration. Cartilage tissues with a high Mankin score were characterized by strong plant lectin staining indicating the expression of functional glycan epitopes in degenerated cartilage. Moreover, binding sites for Galectin-1 were detected in OA cartilage. Importantly, a significant correlation was revealed between the number of Galectin-1 positive chondrocytes and the degeneration score (r=0.71). RT-qPCR and ELISA assays demonstrated that Galectin-1 strongly upregulated Interleukin-1ß as well as Matrix metalloproteinase-3 and -13 mRNA and protein levels in cultured chondrocytes.
Conclusions: Viewing OA from a glycobiological perspective, we found that the expression of glycan epitopes was significantly more pronounced in highly degenerated OA cartilage as compared to intact cartilage. Of note, the expression of Galectin-1 by chondrocytes correlated with the cartilage degeneration score, indicating the potential translation of the sugar code into biological effects. In this context, we demonstrated Galectin-1 binding sites in OA cartilage and showed that Galectin-1 induced catabolic processes in chondrocytes in vitro. In summary, this study suggests a role for altered glycan profiles and Galectin-1 expression in OA disease progression.

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CARTILAGE DAMAGE IN VIVO AND IN VITRO REGULATES MOLECULES THAT DRIVE PAIN IN MURINE OSTEOARTHRITIS

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Purpose: Pain is the main symptom of osteoarthritis (OA), but it is currently unknown which tissues give rise to pain and what drives it in OA. Our previous studies in painful behaviour in mice following surgical induction of OA revealed that mice develop pain at around 8 weeks post-surgery. At this time there is moderate cartilage damage, but little synovitis in the joint. The aim of this study was to identify which pain pathways are activated in painful OA joints, in which tissues are these pathways activated, and what is driving them.
Methods: Partial meniscectomy or sham operation was performed in 10 week old female C57B6 mice. Pain assessments were performed weekly. RNA was isolated from whole knee joints, or from micro-dissected tissues (articular cartilage, meniscus and tibial epiphysis). For cartilage injury responses in vitro, RNA was isolated from hip epiphyses of 5 week old mice, at 0 and 4h post-explantation. RT-PCR was performed using Taqman® Low Density Arrays for 67 genes that are involved in inflammation and pain.
Results: Pain developed 8 weeks after partial menisectomy. Bradykinin receptors (BDKRB1 and 2), protachykinin (TAC1), tachykinin receptor 1 (TACR1), nerve growth factor (NGF), neuropeptide Y (NPY) and CCL21 were the only genes upregulated in the whole joint compared to sham operated joints from the panel of 67 genes. When we examined the microdissected tissues we found that the same genes, apart from BDKRB2 and NPY, were upregulated in cartilage. BDKRB2 was upregulated in the epiphysis. NPY was not regulated in any of the microdissected tissues. To establish whether cartilage injury per se could induce these same genes, we looked at the regulation of pain related genes in explanted murine hips. NGF, BDKRB1/2, TAC1 and NPY were strongly regulated upon cartilage injury in vitro.
Conclusions: This study has revealed a number of important observations:

- A select group of pain mediators and receptors are regulated in the joint when meniscectomised mice develop pain.
- Expression of these mediators and receptors is occurring in a non-inflamed joint, and is not associated with expression of other inflammatory molecules.
- The majority of these pain mediators and receptors are expressed in the damaged cartilage in vivo and regulated by cartilage injury in vitro.

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THE MOLECULAR RESPONSE TO ACUTE JOINT DESTABILISATION IS ALTERED IN FEMALE COMPARED TO MALE MICE.

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Purpose: It is well established that gender is an independent risk factor for the development of osteoarthritis (OA). Human epidemiological studies have highlighted the increased incidence of disease in women after the age of 50 years. This postmenopausal increase in OA has been attributed to changes in sex hormones, and has led to the suggestion that estrogen has chondro-protective effects. This is supported by the observation that the disease severity of surgically induced OA in female pre-menopausal mice is significantly lower than in males, and that in female mice it is increased following oophorectomy. The effect of sex hormones on OA severity could be at the level of the joint tissues themselves, or could be indirect by affecting other factors such as levels of physical activity. The aim of this project was to investigate the mechanisms by which gender affects severity of OA.
Methods: OA was induced in 10 week old male and female C57B/6 mice by destabilisation of the medial meniscus (DMM). OA was assessed by histological assessment of the joints at 4, 8 and 12 weeks post DMM or sham surgery. Activity levels in mice were assessed using LABORAS (Laboratory Animal Behaviour Observation Registration and Analysis System) which is able to distinguish different types of activity (running, eating, climbing, resting). Pain assessments were performed using The Linton incapacitance tester (which measures differential weight born through the operated compared to non-operated joints). RNA was extracted from mouse knee joints for RT-PCR analysis 6h following surgery to determine whether gender influences the molecular response to acute joint destabilisation.
Results: Histological analysis confirmed previous studies demonstrating an increase in severity of OA in male compared to female mice at 4, 8 and

12 weeks post-surgery. This increase in disease was not associated with increased activity. In fact LABORAS revealed that female mice (at 2 weeks post-surgery) spent more time running and climbing compared to their male counterparts. Female mice developed painful behaviour at a similar time post-surgery (12-14 weeks) to males, despite being at an earlier stage of cartilage damage. We have recently described the molecular response to acute joint destabilisation which reveals the mechanosensitive induction of a number of inflammatory response genes and potential repair factors. When we studied this panel of 47 genes in male and female joints we found that some responded identically in male and female joints, whilst other were more highly expressed in the female joints. Specifically, there was no difference in the levels of Adamts5, IL1b or Mmp13, but TIMP1 (the metalloproteinase inhibitor), inhibin (a TGFβ family member), versican and Mmp3 were significantly higher in female joints.

Conclusions: Pre-menopausal female mice have significantly less severe OA compared to males, and this cannot be attributed to higher levels of activity in male mice. Nor is it related to increased painful behaviour as female mice have similar levels as male mice but with less cartilage damage. The response to acute joint destabilisation reveals an increase in predominantly matrix, repair and anti-inflammatory genes in female mice compared to males, thereby suggesting that female mice may mount a more anti-inflammatory/repair response compared to males.

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SUSTAINED DELIVERY OF IL-1RA FROM PLGA MICROSPHERES ATTENUATES IL-1β MEDIATED INFLAMMATION IN A CARTILAGE TISSUE ANALOG

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Purpose: In healthy articular cartilage, chondrocytes maintain equilibrium between degradation and synthesis. Injury, aging, and misuse can disturb this balance triggering degeneration characteristic of osteoarthritis. Current therapeutic options are limited. This study evaluated a novel therapeutic that targets a cause of degradation, inflammatory cytokines using a cartilage tissue analog (CTA). Using IL-1ra delivered by poly(lactic-co-glycolic acid) (PLGA) microspheres (MS) our therapeutic approach mitigates IL-1β's deleterious effects on cartilage.

Methods: *Microsphere Fabrication and Release Kinetics:* PLGA MS were fabricated using the water-oil-water double emulsion technique. Release kinetics were determined in PBS under agitation at 37°C. Over the course of 5 weeks, supernatant was collected and IL-1ra content was quantified using the bicinchoninic acid assay. *Cell Isolation and CTA Fabrication:* Chondrocytes were isolated from juvenile bovine knees. Articular cartilage was removed and minced, and digested with collagenase. The cell suspension was filtered and washed. Cells were seeded 1x107 cells/well into a 96-well plate onto poly (2-hydroxyethyl methacrylate to maintain their cartilage phenotype then cultured for 10 weeks. *Cell Treatment:* The treatment groups were: untreated, 10 ng/ml IL-1β, and 10 ng/ml IL-1β + 500 μg/ml IL-1ra MS. CTAs were harvested after 3 and 6 days of treatment. The 6-day treatment group included 60% media changes at 3 days, with additional doses of IL-1β added to the relevant groups. *mRNA Levels:* RNA was isolated from the CTAs via two sequential extractions using TRIZOL-chloroform (n=6). Reverse transcription was performed on 1 μg of RNA in a 20 μl volume. Quantitative PCR was performed to determine mRNA levels of collagen II, aggrecan, COMP, IL-6, iNOS and ADAMTS4, and normalized to GAPDH. *Nitrite Concentration:* Nitrite levels in the medium were measured using the Griess assay. *Statistics:* Differences between treatment groups were established using ANOVAs with post-hoc Tukey's tests; $p<0.05$ was considered significant.

Results: IL-1ra was released from the PLGA MS over the course of 5 weeks. After a burst release in the first hour (20.2±4.4ng per μg of MS), IL-1ra release leveled to a linear release from day 2 to day 35 of 0.2 ng per μg of MS per day (r2=0.99). Delivering MS to IL-1β treated CTAs inhibited IL-1β's effect. While IL-1β caused a large increase in nitrite concentration simultaneous IL-1raMS delivery reduced the increase. After both 3 and 6 days, CTAs treated with IL-1β had significantly higher iNOS, ADAMTS4, and IL-6 mRNA levels compared to untreated CTAs. IL-1β treated CTAs had a corresponding decrease in the mRNA levels of collagen II, aggrecan and COMP. CTAs treated with both IL-1β and IL-1ra MS had no significant differences compared to the untreated.

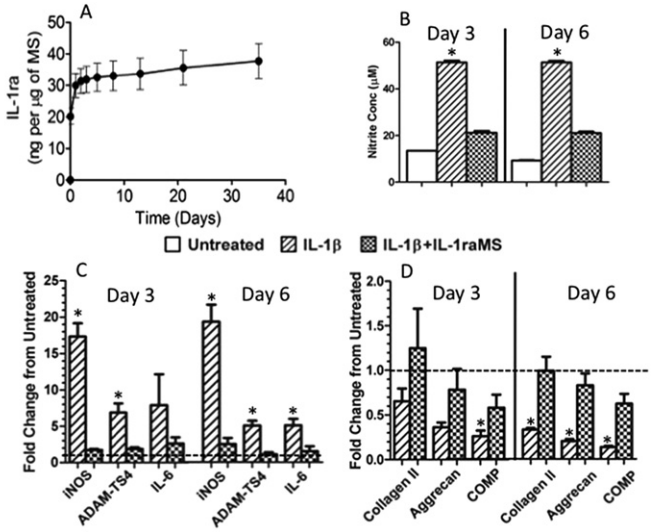

Figure 1: IL-1ra release (a), nitrite concentration (b), catabolic mRNA levels (c) and anabolic mRNA levels (d). *p<0.05 compared to untreated

Conclusions: In this study we showed for the first time that sustained IL1-ra released from PLGA microspheres can effectively inhibit IL-1β mediated inflammation in cartilage. Our results suggest that this inhibition was effective beyond the initial burst release, extending up to 6 days in the presence of repeated doses of IL-1β. This study provides the foundation for developing a novel treatment for osteoarthritis.

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FATTY ACIDS MODULATE DESTRUCTIVE AND INFLAMMATORY PROCESSES IN OSTEOARTHRITIC CARTILAGE

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Purpose: Onset and progression of osteoarthritis (OA) are associated with obesity. Since not only knee OA but also hand OA is associated with obesity, it is plausible that next to increased joint loading, systemic metabolic alterations such as adipose-related inflammation might explain this association. In addition, obesity is correlated with altered fatty acid profiles in serum. Recent studies indicate that n-3 poly unsaturated fatty acids (PUFA's) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decrease inflammation and n-6 PUFA arachidonic acid (AA) increases inflammation in OA joints. In this study we aim to investigate whether other fatty acids known to be present in the knee joint such as linoleic acid (n-6), oleic acid (n-9), and palmitic acid (saturated) are also able to influence cartilage and thereby contribute to processes seen in OA.

Methods: Cartilage was obtained from OA patients undergoing total knee arthroplasty and either cultured as explants (3 donors) or as chondrocytes (2 donors) isolated from the cartilage (passage 3). All were cultured in DMEM low glucose and 1% ITS with or without 10 ng/ml TNFα as a pro-inflammatory stimulus and in absence or presence of DHA, linoleic acid, oleic acid or palmitic acid. Concentrations were determined using mass spectrometry. Analysis of cell cultures included quantification of intracellular lipid deposition using Oil-red-O, lactate hydrogenase (LDH)-assay for cytotoxicity and gene expression for cyclooxygenase 2 (COX2), matrix metalloproteinase (MMP) 1, 3 and 13 and A Disintegrin and Metalloproteinase with ThromboSpondin motifs (ADAMTS) 4. Cartilage explants were analysed for glycosaminoglycan (GAG) release and nitric oxide (NO) production. Independent sample T-test was used for all data analysis.

Results: Culturing chondrocytes in the presence of the fatty acids resulted in an increase of intracellular lipid deposition as determined with Oil-red-O staining. This effect was independent of TNFα presence in the culture